JOURNAL OF CLINICAL MICROBIOLOGY, June 2005, p. 2602–2607 Vol. 43, No. 6
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.6.2602–2607.2005

Characterization of *Clostridium botulinum* Strains Associated with an Infant Botulism Case in the United Kingdom

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Received 16 July 2004/Returned for modification 29 August 2004/Accepted 5 February 2005

The sixth case of infant botulism in the United Kingdom was reported in 2001. The case was caused by a type B strain of *Clostridium botulinum*. Strains of *C. botulinum* were isolated from the baby's feces and from foodstuffs in the household in an attempt to document transmission. The aims of this study were to characterize the strains of *C. botulinum* associated with the botulism case. This was performed using a variety of techniques, including *C. botulinum* culture phenotypic properties, neurotoxin characterization, and pulsed-field gel electrophoresis (PFGE) banding patterns. Cultures associated with this case as well as isolates from stored and historical samples were analyzed and compared. *C. botulinum* type B PFGE patterns from the infant and from an opened container of infant formula were indistinguishable, while the PFGE profile of a strain presumably isolated from an unopened archival container was unique. The results suggest that the unopened brand of formula was not the source for transmission of spores to the infant and that the strain was distinct from previous botulism cases in the United Kingdom. Since environmental testing was not performed, it is not possible to deduce other sources of transmission.

In the United Kingdom infant botulism has been particularly rare, with only five previous confirmed single cases occurring in 1978 (1, 45), 1987 (2, 43), 1989 (29, 41), 1993 (3, 16), and 1994 (4, 5, 17). A sixth case of infant botulism, the subject of the present study, was confirmed in 2001 (6, 7). An isolated case of infant botulism in a predominantly breast-fed weaning 5-month-old infant was reported to the Public Health Laboratory Service (PHLS) (London) (6, 7). There were no obvious predisposing factors within the household, and there was no proximity to a farm, although the case occurred near a new house under construction and disruption of soil and formation of dust could lead to spread of *Clostridium botulinum* spores. Strains of *C. botulinum* type B were isolated from the baby's feces and from an opened container of powdered infant formula in the household by researchers in the United Kingdom. A *C. botulinum* strain was also presumptively isolated from an unopened container of powdered formula. This container was from a manufacturing lot of 122,388 cans in October 1998 and was therefore near the 3-year expiration date. This lot had been distributed throughout the United Kingdom. To our knowledge, the lot had not led to previously reported infant botulism cases. Five sealed retained archival containers of infant formula were submitted for examination by the manufacturer, one of which was reported to contain *Clostridium botulinum* type B. The case was suspected to be associated with the consumption of this source of infant formula. The linking of this case at the time with powdered infant formula led to considerable publicity as well as withdrawal and recall of product and of "sister" batches of the same lot from the shelves. It also spurred the company to conduct a thorough review of manufacturing procedures and quality control.

This infant botulism case was investigated in a collaborative effort by two independent laboratories utilizing microbial culture and molecular techniques for identification of the organism causing the incident and its suspected relation to infant formula. A blinded investigation into the genetic relatedness of the strains from the infant, the opened container and the closed container, as well as historical isolates, was performed using two typing methods: (i) amplified fragment length polymorphism (AFLP) performed at the PHLS in London (unpublished data) and (ii) pulsed-field gel electrophoresis (PFGE) at the University of Wisconsin—Madison in the United States. This communication presents analyses of the various *C. botulinum* strains isolated during the case by PHLS and from retrospective clinical and food isolates using PFGE. Finally, potential measures to investigate and prevent future cases of infant botulism are described.

MATERIALS AND METHODS

Bacterial strains. Twelve lyophilized samples (no. 1 to 12) received blinded from the PHLS were aseptically rehydrated in sterile TPGY media (50 g/liter trypticase peptone, 5 g/liter Bacto peptone, 4 g/liter D-glucose, 20 g/liter yeast extract, 1 g/liter cysteine-HCl, pH 7.4). The TPGY medium was sparged with nitrogen gas prior to autoclaving, and resazurin (2 mg per liter) was used to ascertain anaerobic conditions. These cultures were then grown in TPGY broth and in TPGY supplemented with cooked meat particles (75 g/liter) (TPGY/CM). Cultures were grown at both 30° and 37°C. Since all cultures were determined to be proteolytic, they were grown at 37°C in subsequent experiments. All bacterial

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media components were purchased from Becton Dickinson Microbiology Systems, Becton Dickinson and Company, Sparks, MD.

Cultural properties of *Clostridium botulinum* isolates. Bacterial cultures were examined microscopically to evaluate their purity. All cultures were also grown on TPGY agar plates and incubated aerobically and anaerobically in an anaerobic chamber (with a gas mix containing 80% nitrogen–10% carbon dioxide–10% hydrogen) to determine their purity. Mixed cultures (samples 1, 6, 10, 11, and 12) were heat shocked and/or ethanol treated and then plated on *Clostridium botulinum* isolation media (CBI; 40 g/liter trypticase peptone, 5 g/liter Na_2HPO_4, 2 g/liter NaCl, 2 g/liter D-glucose, 5 g/liter yeast extract, 20 g/liter agar, 100 ml/liter egg yolk suspension, 50% 250 mg/liter cycloserine, 76 mg/liter sulfamethoxazole, and 4 mg/liter trimethoprim) in an attempt to isolate pure cultures. Colonies that were determined to contain rod-shaped bacteria were reisolated from the anaerobic plates and grown in TPGY media for further analyses. Stock cultures were prepared from all purified strains. Proteolytic properties of the pure cultures were determined by examination of degradation of cooked meat particles during growth. After 5 days of growth in TPGY the culture pH was measured.

Botulinum neurotoxin A or B detection and serotyping. Neurotoxin titers in culture supernatants were determined using an intravenous injection method as previously described (32). Culture supernatants obtained from 5 days of growth in TPGY were diluted 1:2 in gel-phosphate buffer (30 mM sodium phosphate buffer, pH 6.3, 0.2% gelatin). One-hundred microliters of each sample were injected into the lateral tail veins of five mice (female ICR mice, 18 to 22 g [Sprague Dawley, Madison, WI]). The animals were observed for characteristic signs of botulism, and the time to death was recorded. The average time to death (ATTD) for 5 animals was converted to intraperitoneal (i.p.) 50% lethal dose (LD_{50})/ml using the formula i.p. $LD_{50}/ml = 6.9 \times 10^6 - 3.4 \times 10^5(ATTD) + 5.7 \times 10^3(ATTD)^2 - 31.9(ATTD)^3$. This assay has been demonstrated in our laboratory to correlate with the mouse i.p. bioassay (32).

Toxin serotype was determined by enzyme-linked immunosorbent assay (ELISA) using cell-free culture supernatants. Cultures were tested with *C. botulinum* type A and type B antitoxins. Five-hundred microliters of culture samples were centrifuged for 5 min at 5,000 × *g* to pellet cells, and the supernatants were used for assays of neurotoxin protein. Ninety-six-well microtiter plates (Corning International, Corning, NY) were coated overnight at 4°C with 100 μl of a 5-μg/ml solution of antibodies raised against highly purified BoNT/A or BoNT/B toxoid. Antibodies were purified by affinity chromatography as previously described (22), diluted in 100 mM sodium carbonate buffer, pH 9.6, and used for coating plates. Plates were washed with TBST (10 mM Tris-HCl, 0.1% bovine serum albumin [BSA; Sigma], 150 mM NaCl, 0.05% Tween 20, pH 7.4) to remove unbound capture antibody, and then treated with 300 μl of blocking solution (5% vitamin-free casein [Research Organics, Inc., Cleveland, OH] in 10 mM Tris-HCl, 0.9% NaCl, 6 mM NaOH, pH 7.6) for 1 h at room temperature. Blocking solution was removed, 100 μl of serial dilutions of culture supernatants and a botulinum neurotoxin type A and B complex standard in blocking solution were added to the microtiter plate wells, and the plates were incubated for 2 h at room temperature. Plates were washed with TBST, 100 μl of a 2-μg/ml solution of biotinylated botulinum neurotoxin type A or B affinity purified immunoglobulin G (IgG) in blocking buffer was added to the microtiter plate wells, and the plates were incubated for 1 h at room temperature. Plates were washed with TBST to remove unbound secondary antibody, 100 μl of a 1:10,000 dilution of an avidin alkaline phosphatase conjugate (ExtrAvidin; Sigma) in blocking buffer was added to the microtiter plate wells, and the plates were incubated for 1 h at room temperature. Plates were again washed with TBST and developed by addition of Gibco BRL ELISA Amplification System reagents (Life Technologies, Rockville, MD) according to the manufacturer's instructions. Plates were read at 490 nm in a SpectraFluor plate reader (Tecan U.S., Inc., Durham, NC). All analyses were conducted in triplicate.

PFGE. PFGE was performed to compare the genomic DNA patterns of the 12 PHLS isolates and well-characterized *C. botulinum* type A1 strains 62A, ATCC 3502, Hall A-*hyper*; type A2 strains Kyoto F and FRI-H1A2; and type B strains Okra B (proteolytic) and 17B (nonproteolytic) from our laboratory collection. Of particular importance to this study, strains Kyoto F and FRI-H1A2 are A2 isolates associated with infant botulism cases in Japan and the United States, respectively.

Bacterial cultures were grown anaerobically in Bellco anaerobic tubes in 10 ml of TPGY media until the optical density at 600 nm (OD_{600}) reached approximately 0.6. Bacterial cells were fixed with formaldehyde to inhibit DNase activity (21). One milliliter of formaldehyde solution was injected through the septum into the 10-ml bacterial cultures. The contents were mixed, and tubes were incubated on ice for 30 min. The fixed bacterial cultures were then transferred to 15-ml centrifuge tubes, and cells were precipitated by centrifugation. Cell pellets were washed twice with 10 ml of 0.85% NaCl solution, resuspended in 1.5 ml of solution containing 10 mM Tris-HCl, pH 8.0, 1 M NaCl, and 100 mM EDTA and was incubated for 10 min in a 50°C water bath. Equal volumes of the cell suspension and 1.5% InCert agarose (BioWhitaker Molecular Applications, Rockland, ME) were mixed, and the cell-agarose mixtures were loaded into plug-forming molds. The gel molds were chilled in a refrigerator for 15 min, and agarose plugs were removed and incubated in 4 volumes of lysis buffer (10 mM Tris-HCl, pH 8.0, 1 M NaCl, 100 mM EDTA, 0.5% Brij 58, 0.2% deoxycholate, 0.5% sarcosyl, 100 μg/ml RNase A, 5 mg/ml lysozyme, 40 U/ml mutanolysin) at 37°C for 12 h. The lysis buffer was removed, and the plugs were rinsed with 50 mM EDTA solution to remove lysozyme, RNase and mutanolysin. Two volumes of PK buffer (10 mM Tris-HCl, pH 8.0, 0.5 M EDTA, 1% sarcosyl, and 1 mg/ml Proteinase K) were added, and the agarose plugs incubated for 48 h with gentle agitation in a 50°C water bath. Following Proteinase K treatment, the plugs were washed with TE buffer and incubated for 2 h at 37°C with shaking in TE buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF) to inactivate Proteinase K. The agarose plugs were washed three times for 2 h each with 10 volumes of TE buffer to remove PMSF. Restriction digests of PFGE plugs were performed with four restriction endonucleases (NruI, SacII, SmaI, and XhoI), and samples were separated using a clamped homogenous electric field system (CHEF-DRII; Bio-Rad, Hercules, CA) following the manufacturer's instructions.

RESULTS

Culture purification. Twelve lyophilized culture samples (no. 1 to 12) received blinded from the PHLS were aseptically rehydrated with sterile TPGY and grown in TPGY/CM medium. Cultures were plated on TPGY and incubated aerobically and anaerobically to assess their purity. Cultures grown in liquid media and on plates were examined microscopically to also evaluate purity. Five of the cultures, nos. 1, 6, 10, 11, and 12, were mixed cultures and contained rods and cocci. In order to isolate pure strains of these bacterial cultures, two approaches were used. The cultures were heat shocked or treated with 50% ethanol (23). The cultures were plated on CBI plates, on which *C. botulinum* colonies form a characteristic opaque zone surrounding them, indicating lipase activity. Colonies that contained rod-shaped bacteria were isolated from the anaerobic plates and grown in TPGY media for further analyses. Stock cultures were prepared from all purified strains.

***C. botulinum* growth characteristics.** The *C. botulinum* strains were analyzed for phenotypic properties. These properties are summarized in Table 1. The purified cultures all had a rod morphology, which varied in length and width dependent on the strain. Certain cultures produced high spore titers, while others sporulated infrequently. The spores varied in size and morphology depending on the strain. After 5 days of growth in TPGY, the final pH values ranged from 5.9 to 6.6 due to the production of organic acids during fermentation. The cultures were all proteolytic but varied markedly in proteolysis, as evaluated by digestion of meat particles in TPGY/CM. All strains showed lipase activity on CBI. These results indicate that there was considerable variation among the strains in cultural properties.

Botulinal neurotoxin production and typing. The strains were evaluated for botulinum neurotoxin production and toxin serotype by ELISA, mouse bioassay, and neutralization of mouse toxicity by serotype-specific antitoxins (23). Except for culture 7 (nontoxinogenic control), all the strains produced either A or B botulinum neurotoxin. None of the toxic supernatants reacted with both A and B antitoxins. Biological toxicity was determined using the mouse LD_{50} bioassay. Cultures 2 and 5 produced type A neurotoxin, with titers of 1.3×10^5 and 5.6×10^5 mouse LD_{50}/ml, respectively. The remaining

TABLE 1. Summary of *C. botulinum* cultures received from the PHLS

PHLS culture no.	Microscopic evaluation of the initial culture (TPGY tubes, 48 h)	Microscopic evaluation of CBI plates (7 days)	Toxicity (5-day culture) (LD_{50}/ml)	Toxin type (ELISA)	pH of 5-day culture	Digestion of cooked meat particles (5-day culture)	PFGE type
1	Mixed culture; large rods and cocci; few spores	Medium rods; few spores	1.5×10^5	B	5.90	+	Group 2
2	Pure culture; large/medium rods; no spores	Medium/large rods; many large spores	1.3×10^5	A	6.40	+	Group 1
3	Pure culture; medium rods; many large spores	Medium rods; many very large spores	2.3×10^4	B	6.44	+	Unique
4	Pure culture; large rods; few spores	Medium rods; many very large spores	1.6×10^5	B	6.61	+++	Group 3
5	Pure culture; long rods; many large spores	Medium rods; many large spores	5.9×10^5	A	6.31	+++	Group 1
6	Mixed culture; long rods and cocci; no spores	Large rods; no spores	3.6×10^4	B	5.89	+	Group 2
7	Pure culture; long rods; many large spores	Medium/large rods; many large spores	None detected	None detected	6.39	++	Unique
8	Pure culture; long rods; many medium sized spores	Large rods; many large spores	2.6×10^5	B	6.49	+	Group 3
9	Pure culture; long rods; many spores	Medium rods; many very large spores	2.6×10^5	B	6.59	++	Unique
10	Mixed culture; medium rods and cocci; no spores	Medium rods; many small spores	2.4×10^4	B	6.27	+	Group 2
11	Mixed culture; long rods and cocci; few spores	Medium rods; many medium-sized spores	1.3×10^4	B	6.56	+++	Unique
12	Mixed culture; long rods and cocci; few spores	Medium rods; many small spores	$\sim 3 \times 10^3$	B	6.45	+++	Unique

nine toxic cultures produced type B neurotoxin, and the titers varied from $\sim 3 \times 10^3$ to 2.6×10^5 mouse LD_{50}/ml.

PFGE analyses. PFGE was used to assess genetic relatedness among the PHLS isolates and well-characterized *C. botulinum* type A1 strains 62A, ATCC 3502, Hall A-*hyper*; type A2 strains Kyoto F and FRI-H1A2; and type B strains Okra B (proteolytic) and 17B (nonproteolytic) from our laboratory collection. Restriction digests of PFGE plugs were performed with four restriction endonucleases: NruI, SacII, SmaI, and XhoI. The digests were separated using a clamped homogenous electric field system. PFGE revealed that some of the isolates showed banding similarities, and we were able to assign them to arbitrary groups (Table 1, Fig. 1). PFGE group 1 included the PHLS isolates 2 and 5, which revealed identical banding patterns in all four digests. Interestingly, these PFGE patterns were identical to the patterns from the infant type A2 botulism-associated strains Kyoto F and FRI-H1A2 (with minor differences in NruI banding pattern). PFGE group 2 included PHLS isolates 1, 6, and 10, which yielded similar banding patterns. The SacII and NruI digestion patterns were identical for these three isolates, while isolates 1 and 10 showed identical SmaI digestion patterns and isolate 6 had a slightly different pattern. All three isolates yielded very similar patterns by XhoI, with minor differences in some band sizes. The banding pattern of these isolates (1, 6, and 10) did not reveal similarity to any of the type A or B *C. botulinum* strains analyzed. PFGE group 3 included PHLS isolates 4 and 8, which had identical banding patterns with all four restriction endonucleases. The patterns did not show any similarity to other *C. botulinum* strains examined. The PHLS isolates 3, 7, 9, 11, and 12 exhibited unique PFGE patterns with no similarity to the other PHLS isolates or *C. botulinum* type A and B strains from our collection used for comparison.

After these cultural, toxin, and PFGE analyses were completed, the strain origins were unblinded (Table 2). It was revealed that strains 1, 6, 10, 11, and 12 were isolated during the investigation of the infant botulism case in 2001. All of these strains produced type B botulinum neurotoxin. Isolates 1 (from the baby's feces) and isolates 6 and 10 (from opened containers of baby milk) had the same PFGE pattern (Group 2). However, isolate 6 differed from isolates 1 and 10 in cell morphology, spore titers, and toxin production in culture. Isolate 11 (from the baby's feces) produced type B toxin but had a unique PFGE pattern which did not show similarity to the other strains examined. Strain 12 (presumptively isolated from an unopened infant formula) also produced low levels of type B toxin, was strongly proteolytic in comparison to strains 1, 6, 10, and 11, and importantly had a unique PFGE pattern with no similarities to the other strains. Interestingly, isolates 2 and 5 (from different United Kingdom infant botulism cases), Kyoto F associated with an infant botulism case in Japan, and FRI-HIA2 from honey associated with an infant botulism case in the United States all produced type A toxin and had identical PFGE patterns. These results indicate that cultural, toxin, and PFGE analyses can clearly distinguish groups of *C. botulinum*, even from widely different geographic regions of the world.

DISCUSSION

Since the discovery of infant botulism in 1976 (34, 38), infant botulism has been detected very rarely in the United Kingdom.

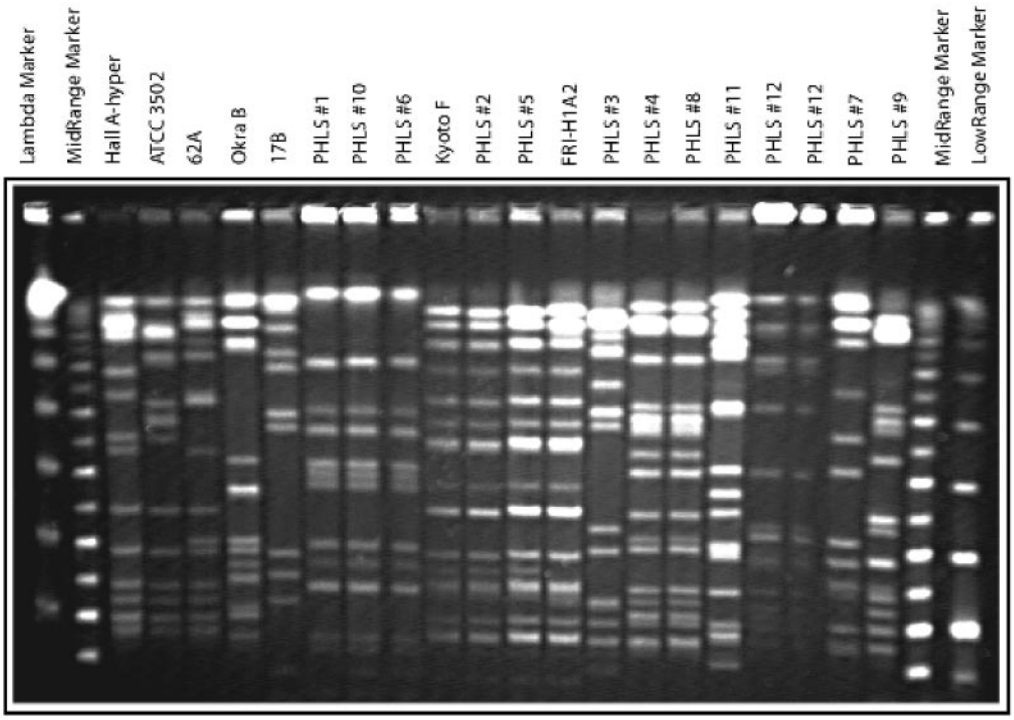

FIG. 1. Representative PFGE results: digestion patterns of *C. botulinum* strains using restriction enzyme NruI. The pulse-time ramp was 5 to 20 s, and the running time was 23 h. See Materials and Methods for details.

This study describes an investigation of the sixth case that occurred in 2001. Infant botulism is caused by the ingestion of spores of *C. botulinum* followed by gut colonization by the organism and absorption of the toxin into the blood (19, 34, 38). Epidemiological risk factors for infants include rural residence where agricultural practices are carried out and urban areas where building or earth moving for road construction are frequent (10). Ingestion of honey (10) or possibly corn syrup (36) are also risk factors. Although the predominance of cases of infant botulism recorded to date have occurred in the state of California, infant botulism has also been reported in other regions of the United States (8, 35), Italy (20), Denmark (12), Japan (30), Venezuela (46), Australia (33), and various other countries (10). Some reports suggest a link with sudden infant

TABLE 2. Origins, toxin serotypes, and PFGE groups of the strains used in this study[a]

Sample group and PHLS culture number	FSML reference no.	Source	Toxin type	PFGE group
Stored and historical isolates				
7	F2641/89	Routine fecal specimen, 1989 *Clostridium* spp. (not *C. botulinum*)	None	Unique
3	F2534/89	FB 1989, isolated from yogurt (37)	B	Unique
9	R2283H/98	FB 1998, isolated from feces (39)	B	Unique
4	R2045/98	FB 1998, isolated from mushrooms (39)	B	Group 3
8	F3602A/93	IB 1993, isolated from feces (CDSC, 1993)	B	Group 3
2	F638/94	IB 1994, isolated from feces (CDSC, 1994)	A	Group 1
5	R1688/94	IB 1994, isolated from feces (CDSC, 1994)	A	Group 1
Infant botulism incident of 2001				
1	R1349/01 PC6	Isolated from baby's feces	B	Group 2
11	R1349/01 PC5	Isolated from baby's feces	B	Unique
6	R1396/01 H4 PC5	Isolated by FSML from opened infant formula	B	Group 2 (differed slightly from 1 and 10 by SmaI digestion)
10	R1396/01 H4 PC9	Isolated by FSML from opened infant formula	B	Group 2
12	R2113/01	Isolated by CSL from unopened infant formula	B	Unique

[a] Abbreviations: CDSC, Communicable Disease Surveillance Centre; CSL, Central Science Laboratory; FSML, Food Safety Microbiology Laboratory; FB, foodborne botulism; IB, infant botulism.

death syndrome (13) but others do not (15), and the possible relationship of sudden infant death syndrome and infant botulism remains controversial. Although infant formula feeding has occasionally been implicated in the transmission of infections such as salmonellosis and *Enterobacter sakazakii* (9, 11, 18), it has not been epidemiologically linked to infant botulism (10). Large numbers of samples of infant formula were tested for *C. botulinum* spores in the 1980s, and no evidence of contamination with spores of *C. botulinum* was found (S. Arnon, personal communication).

This investigation is unique in that independent studies were performed by two different laboratories, one in the United Kingdom at the PHLS and the other in the United States at the University of Wisconsin—Madison. The two laboratories used distinct genetic approaches to characterize *C. botulinum* strains. The United Kingdom study, which will be published elsewhere (unpublished data), used AFLP to analyze strains isolated during the 2001 infant botulism case. Our study used cultural properties, toxicity, and PFGE analyses to characterize the 2001 isolates as well as other PHLS isolates, some of which were associated with previous food and infant botulism incidents. These strains were received blinded in our laboratory from the PHLS. In addition to the PHLS cultures, several *C. botulinum* type A and B strains from our collection, including the A2 isolates Kyoto F and FRI-H1A2 previously associated with infant botulism, were included in the PFGE analyses.

Various methods have been used for molecular typing of *C. botulinum*, including ribotyping, PCR, repetitive element sequence-based PCR (rep-PCR), random amplified polymorphic DNA analysis (RAPD), amplified fragment length polymorphisms (AFLP), and PFGE (14, 25, 26, 27, 28, 40, 42). In our laboratory, we have found PFGE to be a rapid and accurate method of determining genomic relationships among groups and strains of *C. botulinum* (28, 31). AFLP and ribotyping are less time-consuming and are useful for typing of strains for which satisfactory high-molecular-weight chromosomal DNA preparations and restriction fragment digests cannot be obtained. In our experience as well as in certain other laboratories, PFGE analysis of *C. botulinum* strains has been the most discriminating technique (24, 25, 28). Computer programs have been developed to accurately analyze PFGE data and for molecular typing and disease surveillance for other bacterial pathogens in the PulseNet system (44).

In this study, the two isolates from the infant's fecal specimens differed in PFGE patterns. One isolate from the opened container matched an isolate from the infant's fecal specimen, and one isolate from the unopened container had a unique PFGE pattern. The fact that the strain presumptively isolated from the unopened container differed from those isolated from infant feces and the opened container of infant formula indicates that the source from which the infant was infected is not proven. It is not clear how the opened can was contaminated and the child was exposed, particularly since the home and environs near to the home were not sampled for *C. botulinum* during the investigation.

Since the commercial source of the baby formula was initially suspected to cause the infant botulism case, a large recall was undertaken on the basis that a single strain was presumptively isolated from an unopened container, suggesting an apparent link to a clinical case. Since the batch of this infant formula consisted of 122,388 containers manufactured in October 1998 and fed to around 30,000 infants within the United Kingdom through the 3-year shelf life, if contamination of the batch had been widespread it is likely that numerous cases of infant botulism would have occurred in 1998, but this was not the case. Although proprietary, the general ingredients of the infant formula consisted of reduced mineral whey, vegetable oils, skimmed-milk powder, and lactose. Due to the ubiquity of bacterial spores, these products could be contaminated. One of the processing steps was heat treatment at 90 to 95°C for 20 to 25 s, which would not be sufficient to inactivate heat-resistant bacterial spores. Therefore, it is possible that *C. botulinum* spores could survive the manufacturing process of the infant formula, but this would require a separate study and sampling of a statistically meaningful number of containers to show their presence in infant formula. Although the infant botulism case did not appear to be associated with the commercial "unopened" source of infant formula, nonetheless it stimulated the industry to consider various precautionary measures and actions to prevent infant botulism from powdered formula. Most importantly, measures should be taken to ensure that viable *C. botulinum* spores are not present in baby formula.

This study describes an investigation of the association of *C. botulinum* with an infant botulism case. The investigation utilized various methods, including microbiological analyses, toxin testing and typing, and molecular typing for epidemiological characterization and strain identification of isolates associated with infant botulism. It was evident from this study that typing methods can provide new insights into causation and transmission of infectious diseases, including infant botulism.

ACKNOWLEDGMENTS

We thank Eric Bolton, Moira Brett, Jim McLauchlin, and their colleagues for sending the strains and for the collaborative study.

This study was supported by sponsors of the Food Research Institute and the College of Agriculture and Life Sciences, University of Wisconsin, Madison.

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